UNIVERSITI PUTRA MALAYSIA

EFFECTS OF *Moringa oleifera* HYDEOETHANOLIC LEAF EXTRACT ON THE SERUM LIPID PROFILE IN HYPERCHOLESTEROLAEMIC RATS

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SERDANG, SELANGOR

2018

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A project paper submitted to the
Faculty of Veterinary Medicine, Universiti Putra Malaysia
in partial fulfillment of the requirement for the
DEGREE OF DOCTOR OF VETERINARY MEDICINE
Universiti Putra Malaysia
Serdang, Selangor Darul Ehsan.

MARCH 2018

It is hereby certified that we have read this project paper entitled "Effects of *Moringa oleifera* hydeoethanolic leaf extract on the serum lipid profile in hypercholesterolemic rats", by Loo Xin Yi and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement for the course VPD 4999 – Final Year Project.

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DEDICATION

To my parents and all the cat owners.

ACKNOWLEDGEMENTS

Foremost, I would like to express my sincere gratitude to my advisor Associate Professor Dr. Hazilawati binti Hj. Hamzah for the continuous support of my study, for her patience, motivation, and enthusiasm and knowledge. Her patience guidance helped me in all the time of research and writing of this thesis.

Besides, I would also like to thank my co-supervisors, Dr. Mohd Rosly Bin Shaari for his support and guidance.

My sincere gratitude also goes to both post-graduation students Dr. Aliyu Abdullahi and Miss Syahirah, for helping me greatly in carrying out the study in Animal Metabolism, Toxicology and Reproductive Centre, MARDI, Serdang, Selangor. Without them, this study won't be a success.

To all the staff of Haematology & Biochemistry Laboratory who always lend a kind hand to me whenever I faced difficulty.

To all the experimental rats for their sacrifice in this research, the opportunity to

Not to forget, my comrades Lee Ting-Ying and Khanmani a/p Anpuanandam who had help and motivate me throughout this research.

Last but not least, to my beloved parents who had support and advise me throughout my life.

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Abstrak

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek Akhir Tahun

KESAN ESTRAK HIDROETANOL DAUN *Moringa oleifera* PADA PROFIL LEMAK SERUM TIKUS BERKOLESTEROL TINGGI

By

Loo Xin Yi

2018

Penyelia : Profesor Madya Dr. Hazilawati Binti Hj. Hamzah

Moringa oleifera Lam, (*Moringa pterygosperma*) juga dikenali sebagai ‘drumstick tree’ dan ‘horse radish tree’ di India atau ‘kelor tree’ dalam sesetengah negara merupakan tumbuhan terkenal dengan kesan terapeutiknya. Walaupun begitu, terdapat hanya maklumat yang terhad mengenai aktiviti mengurangkan kolesterol oleh ekstraks daun *Moringa oelifera*. Dalam kajian ini, kesan bagi ekstrak hidroetanol daun *Moringa oleifera* telah dikaji atas tikus yang berkholesterol tinggi diaruh oleh alkohol dan makanan berkolesterol tinggi. Sejumlah 25 ekor tikus telah dibahagikan kepada 5 kumpulan (n=5). Kumpulan A sebagai kawalan neutral diberikan makanan komersial, group B sebagai kawalan negatif diberikan alkohol dan makanan berkolesterol tinggi, kumpulan C, D, dan E sebagai rawatan diberikan alkohol dan makanan berkolesterol tinggi diikuti dengan pemberian ekstak daun *Moringa oleifera* pada dos rendah, sederhana dan tinggi masing-masing. Penilaian dijalankan selepas eksperimen telah tamat dengan analisa parameter hati dan lipid dalam serum. Hasil kajian menunjukkan berat relatif hati kumpulan B (4.11 ± 0.35) adalah lebih tinggi (hepatomegali) dengan signifikan ($p<0.05$) berbanding dengan kumpulan A (3.32±0.46), mempamerkan kajian ini berjaya mengaruhkan tikus yang berkolesterol tinggi. Kajian yang dijalankan selama 27 hari ini juga berjaya mengurangkan berat lemak sekeliling buah pinggang pada kumpulan rawatan C (0.94±0.17), D (0.65±0.41) dan E (1.04±0.52) berbanding dengan kumpulan B. Liporotein ketumpatan rendah (LDL) dalam kumpulan C (0.94±0.17), D (0.65±0.41), E (1.04±0.52) adalah lebih tinggi berbanding dengan kumpulan B (1.25±0.05) dengan signifikan ($p<0.05$). Ekstrak itu juga menunujukan fungsi menurunkan indeks aterosklerotik diaruh oleh alkohol dan diet berkholesterol tinggi seperti yang dipaparkan dalam keputusan kumpulan C (0.27±0.04), D (0.30±0.06), E (0.36±0.07) berbanding dengan kumpulan B (0.41±0.02). Walau bagaimanapun, kajian ini mununjukan tiada signifikan terhadap dos bergantung kepada kesan dalam kumpulan rawatan pada dos yang berbeza. Kesimpulannya, ekstrak daun *Moringa oleifera* dengan ketaranya berjaya mengurangkan hepatomegali dengan mengurangkan trigliserida hati, lemak buah pinggang, LDL serum dan indeks aterosklerotik. Keputusan ini mmencadangkan yang ekstrak daun *Moringa oleifera* ini mempunyai kesan perlindungan hati pada tikus yang berkolesterol tinggi.

Kata kunci: *Moringa oleifera*, LDL, indeks aterosklerotik, tikus berkolesterol tinggi

Abstract

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfillment of the course VPD 4999 – Final Year Project

EFFECTS OF *Moringa oleifera* HYDROETHANOLIC LEAF EXTRACT ON SERUM LIPID PROFILE IN HYPERCHOLESTEROLAEMIC RATS

By

Loo Xin Yi

2018

Supervisor : Associate Professor Dr. Hazilawati Binti Hj. Hamzah

Moringa oleifera Lam, (*Moringa pterygosperma*) also known as 'drumstick tree' and 'horse radish tree' in India or 'kelor tree' in some part of the world is a plant that serve several medicinal and nutritional properties. However, until now, very scanty information is available regarding the anti-hypercholesterolaemic activity of *M. oleifera* hydroethanolic leaf extract. In this study, the effect of *M. oleifera* leaf extract was evaluated on hypercholesterolaemic rats induced with alcohol and high-cholesterol diet. A total of 25 male Spague Dawley rats were randomly divided into 5 groups (n=5). Group A as a neutral control receiving normal commercial diet, group B as a negative control, receiving alcohol and high cholesterol diet without treatment, groups C, D, and E as the treatment group, receiving alcohol and high cholesterol diet, concurrent with the *M. oleifera* hydroethanolic extract at the low, medium and high dose, respectively. At the end of the experiment, blood and liver were collected to measure the serum and liver lipid profile. Liver lipid extraction was done following an adaptation of the published Bligh and Dyer method. Result showed that the relative liver weight of group B (4.11 ± 0.35) was significantly higher compared to group A (3.32±0.46), confirming the negative effect of the alcohol and high cholesterol diet experiment. The 27 days of treatment also significantly reduced kidney fat of groups C and D, compared to group B. The serum low density lipoprotein (LDL) levels of groups C (0.94±0.17), D (0.65±0.41), E (1.04±0.52) were significantly ($p<0.05$) lower compared to group B (1.25±0.05). The extract also significantly ($p<0.05$) reduced the atherosclerotic index induced by alcohol and high cholesterol diet as seen in groups C (0.27±0.04), D (0.30±0.06), E (0.36±0.07) as compared to group B (0.41±0.02). However, there was no significant dose effect observed between the treatment groups of different dosages. In conclusion, administration of *M. oleifera* hydroethanolic extract for 27 days significantly reduced hepatomegaly by decreasing hepatic triglyceride, reduced renal rat, serum LDL and atherosclerotic index. These results suggest that *M. oleifera* hydroethanolic extract has hepatic-protective properties in hypercholesterolaemia rats.

Keywords: *Moringa oleifera*, LDL, atherosclerotic index, hypercholesterolaemic rat.

1.0 Introduction

1.1 Introduction

Feline hepatic lipidosis is one of the most common hepatobiliary diseases recognized in clinical practice (Center *et al*., 1993). In a retrospective prognostic study done by Kuzi *et al*. (2017) showed that the overall motility can reach up to 38%.

From a review study on herbal medicines (Verma *et al*., 2008), has claimed that medicinal herbs had attained a significant role in health system all over the world for both humans and animals as potential source of therapeutics aids not only in the diseased condition but also as potential material for maintaining proper health. *Moringa oleifera* (Figures 1 and 2), regionally known as the horseradish tree or the drumstick tree is widely distributed in the tropical countries (Fahey, 2005). This plant also known as the 'miracle tree' because of all its parts are used for nutritional, pharmacological properties. *M. oleifera* leaf acts as a good source of natural antioxidant due to the presence of various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids (Anwar *et al*., 2007). In another phytochemical study showed that the cholesterol-lowering action of the plant might be attributed to the presence of various bioactive compounds such as chlorogenic acid, rutin, quercetin glucoside, and kaempferol rhamnoglucoside (Arabshadi *et al*., 2007). In this study the effects of *M. oleifera* hydroethanolic leaf extract on the serum liver and lipid profile in hypercholesterolaemic rat model were evaluated.

Figure 1 Leaves of *Moringa oleifera*

Figure 2 Moringa oleifera *trees*

(Source: Tree for life)

1.2 Objectives

The study was undertaken to investigate the *in vivo* effects of *M. oleifera* hydroethanolic leaf extract on alchohol and high cholesterol diet-induced liver cell damage through the analysis of serum biochemistry of liver and lipid parameters.

1.3 Hypotheses

The null hypotheses for this study was:

$H1_{0.}$ There will be no significant differences in the serum liver and lipid profiles of rats fed with alcohol and high cholesterol diet supplemented and non-supplemented with the hydroethanolic extract of *M. oleifera* leaves.

The alternative hypotheses for this study was:

$H1_{a.}$ There will be significant differences in the serum liver and lipid profiles of rats fed with alcohol and high cholesterol diet supplemented and non-supplemented with the hydroethanolic extract of *M. oleifera* leaves.

2.0 LITERATURE REVIEW

2.1 Feline hepatic lipidosis

Hepatic lipidosis has been defined as the accumulation of triglyceride in the liver >5% of the gross liver weight (Pazak *et al*., 1998). Feline hepatic lipidosis can also be characterized by the accumulation of excessive triglycerides (TGs) in more than 80% of the hepatocytes (Valtolina, 2016). According to a retrospective study done by Center *at al*. (1993), feline hepatic lipidosis is one of the most common feline hepatobiliary disorders recognized in clinical practice. Cats of middle-aged adult with the history of over-conditioning followed by inappetence ranging from 2 to 7 days are predisposed to develop hepatic lipidosis (Center, 2005). Physical assessment of most cats showed obvious hepatomegaly, jaundice, dehydration, and a weight loss > 25% of usual body weight. Clinico-pathological features are characterized by hyperbilirubinaemia and increased activities of serum alanine transferase (ALT), aspartate transferase (AST), and alkaline phosphatase (ALP), with only small if any increase in gamma-glutamyl transferase (yGT) activity (Center, 2005). Also, 32% of cats with hepatic lipidosis tested were hypercholesterolemic (Center *et al*., 1993). Blanchard *et al*. (2003) observed that there is a rise in plasma TG, very low density lipoprotein (VLDL) and LDL, and an enrichment of LDL with TG and of high density lipoprotein (HDL) with cholesterol, suggesting that VLDL secretion is enhanced, VLDL and LDL catabolism is lowered, and lipoprotein exchanges are impaired during the course of disease. In the case of idiopathic feline hepatic lipidosis, hepatic accumulation of TG may occur with increased uptake of non-esterified fatty acids (NEFA), impaired fatty acid oxidation, disturbances in VLDL assembly and secretion or disruption in any combination of the above pathways. (Valtolina *et al*., 2016). The lipid and lipoprotein profile of feline hepatic lipidosis showed some degree of similarity with the hypercholesterolaemic state that had been induced in rats in this study.

2.2 Moringa oleifera

Moringa oleifera Lam, (*Moringa pterygosperma*) also known as 'drumstick tree', describing the shape of its pots, and 'horse radish tree' describing the taste of the roots, in India or 'kelor tree' in some part of the world. This plant is widely distributed in the tropics and subtropics region of the world (Baumer, 1983). *Moringa oleifera*, native of the western and sub-Himalayan tracts, India, Pakistan, Asia Minor, Africa and Arabia (Somali *et al*., 1984; Mughal *et al*., 1999) is now distributed in the Philippines, Cambodia, Central America, North and South America and the Caribbean Islands (Morton, 1991). *Moringa oleifera* can be easily found cultivated throughout the plains, especially in hedges and in house yards, thrives best under the tropical insular climate, and is plentiful near the sandy beds of rivers and streams (Anwar *et al*., 2007). The tree can reach the height of 8m with smooth greyish-green bark and has 2-3 pinnate leaves. (Baumer, 1983). Almost all parts of the part serve medicinal and nutritional properties. Leaves, flowers, seeds, pods (drumsticks), roots, bark, gum, and oil (from seeds) each contain numerous different phytochemicals and nutrients (Fahey, 2015). *Moringa* *oleifera* leaves act as a good source of natural antioxidant due to the presence of various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids (Anwar *et al*., 2007). According to Ghasi (2000) the hypolipidaemic potential is associated with the presence of β-sitosterol in crude extract of *M. oleifera*. Report from (Fakurazi *et al*., 2012) proved the antioxidant nature of *M. oleifera* hydroethanolic extracts in acetaminophen-induced hepatotoxicity in rat model. Studies done by (Bais *et al*., 2014) showed that the leaves have antiobesity and hypolipidaemic activity. Hepatoprotective activity of *M, oleifera* against cadmium toxicity in rats is demonstated by Toppo *et al*. (2015). The methanolic extract of the leaves also shown to be effective in the reduction of pain (Manaheji *et al*., 2011). However, until now, very scanty information is available regarding the anti-hypercholesterolaemic activity of *M. oleifera* hydroethanolic extract in an experimental model of high cholesterol diet and alcohol induced liver injury in rats.

3.0 MATERIALS AND METHODS

3.1 *Moringa oleifera* hydroethanolic leaf extract

The freeze-dried extract was mixed with 5% dimethyl sulfoxide (DMSO) to prepare the extract at low, medium and high dose.

3.2 High cholesterol diet preparation

Commercial pellet was ground and mixed with 2.5% cholesterol (3-β-Hydroxy-5-cholestene, Sigma Life Science, Japan). The commercial pellet diet comprised of 21% crude protein, 5% crude fat, 13% moisture, 8% ash, 0.8% calcium and 0.4% phosphorus (Gold Coin-mouse pellet feed). Food intake was calculated every day and body weight was measured once in every week with a digital balance (AND GF-3000, A&D Company, Japan).

3.3 Ethanol (20%) preparation

A volume of 100 mL of 20% ethanol was prepared by mixing 20 ml pure ethanol (99.99%) and 80 mL of distilled water. Each rat from groups B, C, D, E was orally gavage with standardized 0.5 mL/rat of 20% ethanol, approximately at the dosage of 1000 mg/200g(BW)/day.

3.4 Experimental animal

A total of 25 male Sprague-Dawley rats were bought from a local supplier and randomly divided into five groups (n=5). They were acclimatized for 1 week and were placed in propylene cages in Animal Metabolism, Toxicology and Reproduction Center

(AMTREC), Malaysian Agricultural and Research Development Institute (MARDI), Serdang, Selangor, at 22-25ºC, 40-70% humidity, 12 hour dark/12 hour light cycle with standard food and distilled water *ad-libitum*. The protocol of the experiment was approved by the Institutional Animal Ethics Committee with approval number UPM/IACUC/AUP – U007/2018.

3.4 Experimental design

It comprised of two control groups and three treatment groups. The experimental design is tabulated in Table 1.

Table 1. Experimental design.

Group	Alcohol	Diet	*Moringa oleifera* extract
A	Normal saline	Commercial diet	
B	0.5 mL 20% ethanol	Commercial diet + 2.5 % cholesterol diet	
C	0.5 mL20% ethanol	Commercial diet + 2.5 % cholesterol diet	low dose
D	0.5 mL 20% ethanol	Commercial diet + 2.5 % cholesterol diet	medium dose
E	0.5 mL 20% ethanol	Commercial diet + 2.5 % cholesterol diet	high dose

Note: Standardized volume of 0.5 mL ethanol and 0.5 mL *Moringa oleifera* hydroethanolic extract were given daily via oral gavage.

3.5 Sample collection

Feeding habits of all the animals were observed carefully throughout the experimental schedule. On 28th day of experiment, all the rats are euthanized using carbon dioxide chamber. Their blood was then collected from the cardiac puncture with a 5 mL syringe (21-gauge hypodermic needle), and serum samples were separated. Perirenal fat was removed from both kidneys standardized by only one person extracting. Liver and perirenal fat pad mass were weighted on the electrical balance.

3.6 Serum biochemistry

After blood collection, serum samples are separated to analyze liver enzymes (AST, ALT, ALP, GGT), triglycerides, cholesterol, HDL, LDL, albumin, total serum protein, creatinine and urea using an automatic biochemistry analyzer (Biolis 24i Premiun, Abaxis, UK). Globulin levels were calculated by subtracting the albumin from the total protein.

3.7 Statistical Analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 20. All the data are presented as mean ± SEM. Comparison between groups was made by one-way analysis of variance (ANOVA). Statistical significance of the differences between the treatments groups in experiments were analyzed by post hoc test Tukey's multiple comparison tests. Differences between groups were considered significant at $p < 0.05$.

4.0 RESULTS

4.1 Relative liver weight

To reduce the effect of differences in body weight on liver weight comparisons, the data had been normalized to body mass (Figure 3). There was a significant elevation ($p<0.05$) in the relative liver weight observed in group B revealing that there was some degree of hepatomegaly. The reduction trend was observed in the treatment groups, although there were no significant decreases in the relative liver weight results as compared to the negative control (group B).

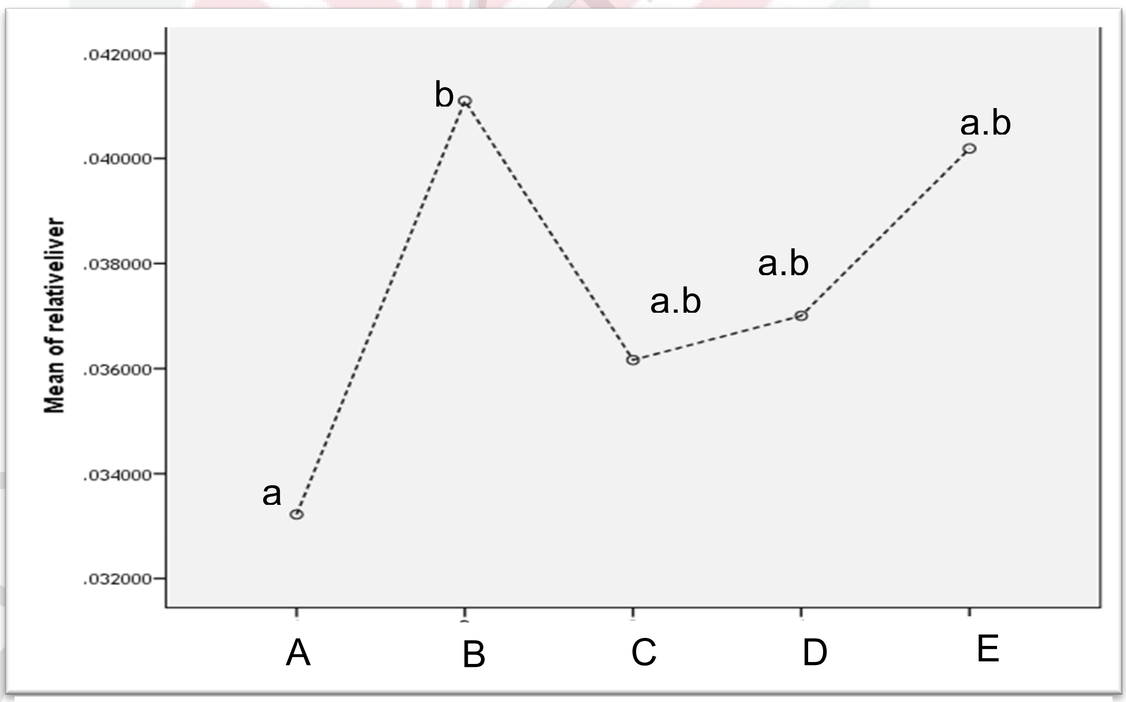

Figure 3 Effect of Moringa oleifera on relative liver weight (mean, N=5), $P<0.05$ (ANOVA followed by post-hoc tukey test). Values with different superscript were significantly different at $p<0.05$

4.2 Relative perirenal fat pad weight

The changes in perirenal fat pad mass after feeding high cholesterol diet and alcohol diet for 27 days are shown in Figure 4. The significant rise ($p<0.05$) up to 136% in relative perirenal fat pad of group B as compared to group A rats fed solely only commercial diet showed that prolonged feeding of high cholesterol and alcohol will eventually cause the accumulation of adipose tissue. As similar trend observed in the relative liver weight, the treatment groups showed no significant result in the reduction of perirenal fat pad as compared to the group B rats.

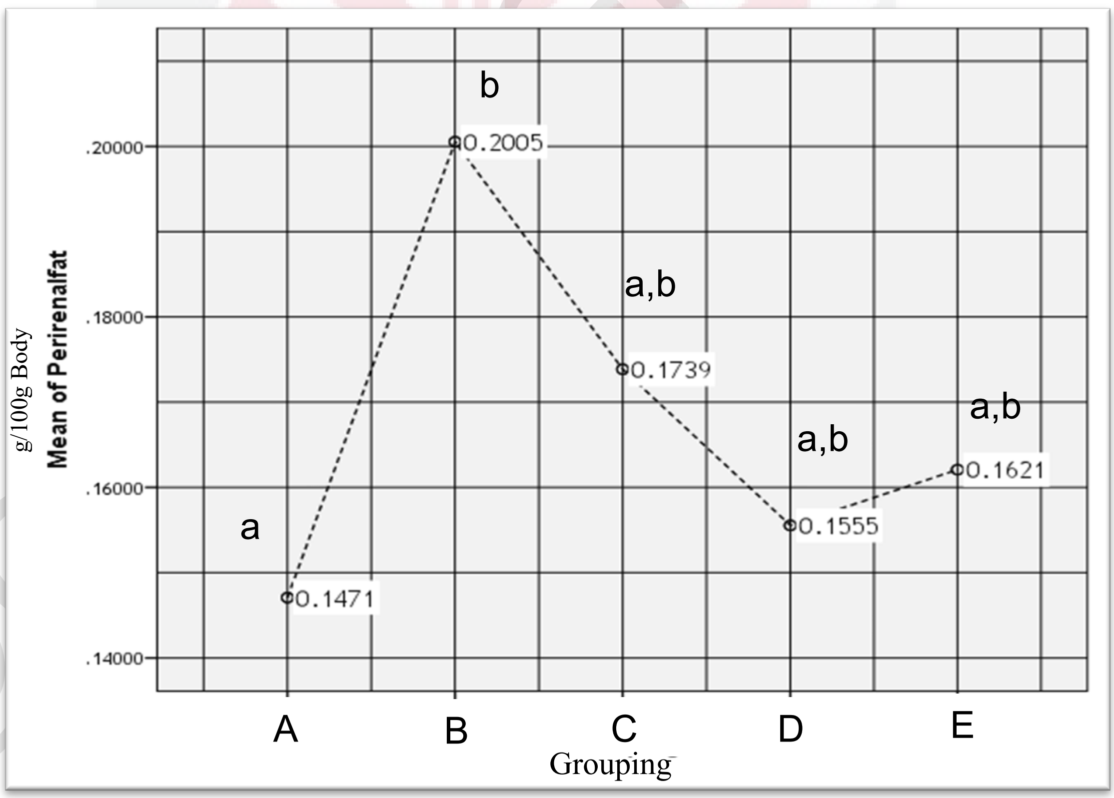

Figure 4 Effect of Moringa oleifera on relative perirenal fat pad mass (mean, N=5), $P<0.05$ (ANOVA followed by post-hoc tukey test). Values with different superscript were significantly different at $p<0.05$.

Results of low density lipoprotein (LDL), indicating hypercholesterolaemia, are shown in Figure 5. There was a significant rise ($p<0.05$) in the serum LDL of group B, more than twice the amount, as compared to group A. The LDL levels decreased significantly by 20.8%, 48.0% and 41.36% when treated with low, medium and high dose respectively with *M. oleifera* hydroethanolic extract. Although there was a reduction in LDL level, treatment of hypercholesterolaemic rats with *M. oleifera* hydroethanolic extract for 27 days was not enough to show a considerable restoration of LDL parameter to that of normal level. The effect of increased serum LDL levels and the formation of perirenal fat pad in the high fat diet-feed group was allied with a significant increase of liver weight.

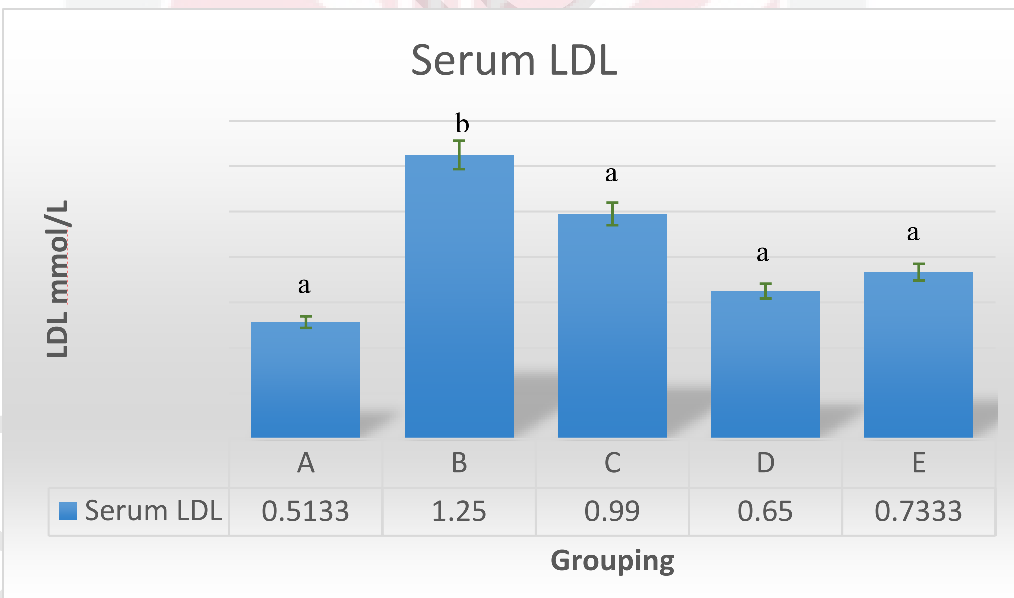

Figure 5 Effect of *Moringa oleifera* on serum LDL (mean, N=5), $P<0.05$ (ANOVA followed by post-hoc tukey test). Values with different superscript letters were significantly different at $p<0.05$.

4.4 Atherosclerotic index

Further calculation was done using LDL, HDL and TC values to obtain the atherosclerotic index (Figure 6), which is regarded as a marker for various cardiovascular disorders. The higher the value, the higher the risk of developing cardiovascular disease and vice versa (Hou *et al*., 2009). Atherosclerotic index showed similar trend as the previous parameters, with significant ($p<0.05$) reduction in the treatment groups. The decreased atherosclerotic index by *M. oleifera* hydroethanolic extract seems to support the cardio-protectant nature of *Moringa oleifera* leaves.

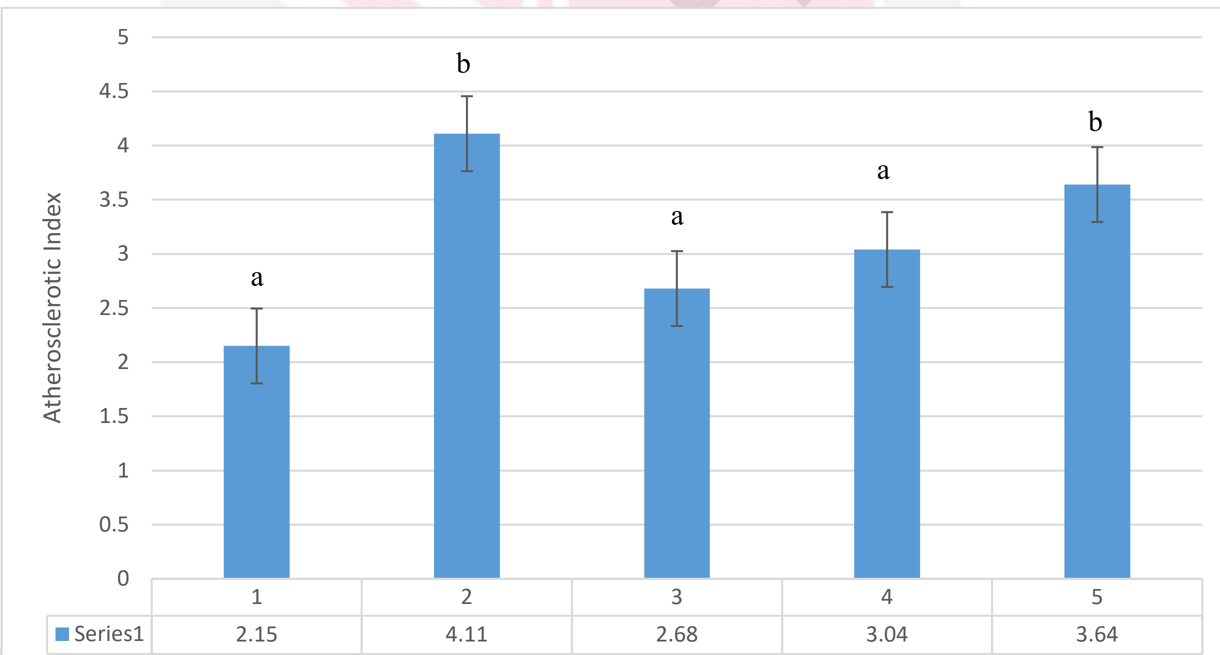

Figure 6 Effect of *Moringa oleifera* on atherosclerotic index (mean, N=5), $P<0.05$ (ANOVA followed by post-hoc tukey test). Values with different superscript were significantly different at $p<0.05$.

Table 1 Summary of significant results. All values are expressed as mean ± SEM, (n = 5), one-way ANOVA, Tukey's post hoc test.

Grouping	Relative liver weight	Perirenal Fat	LDL	AI
A	3.32 ± 0.46	1.44 ± 0.66	0.51 ± 0.33	2.15 ± 0.04
B	4.11 ± 0.35*	2.45 ± 0.89*	1.25 ± 0.05*	4.11 ± 0.12*
C	3.79 ± 0.40	1.74 ± 0.83	0.99 ± 0.16**	2.68 ± 0.19**
D	3.70 ± 0.44	1.34 ± 0.85	0.65 ± 0.41**	3.04 ± 0.33**
E	4.02 ± 0.29	1.91 ± 0.10	0.73 ± 0.22**	3.64 ± 0.33

* $P < 0.05$, versus control group (group A)

** $P < 0.05$, versus negative control (group B)

5.0 Discussion

5.1 Hypercholesterolaemia model

After 27 days of induction, prolonged administration of a high-cholesterol diet and alcohol to the rats in this present study successfully induced the state of hypercholesterolaemia in consistent with the significant elevation in the relative liver weight, relative perirenal fat pad mass, LDL and the atherosclerotic index as shown in the group B rats. This is in agreement with Tan *et al*. (2015) that prolonged feeding of high-cholesterol diet and alcohol will accelerate the hyperlipidemia in the rats, although Tan et al. (2015) managed to induced the hypercholesterolemic state in merely 19 days and at the alcohol dosage of 40 mg/rats (estimated body weight of 200 gm), reaching a 0.2 mg/gm through giving a standardised volume of 0.2 mL/rat. This present study, on the other hand, uses the same 20% ethanol concentration at a higher volume of 0.5 mL/rat reaching 100 mg/rat (estimated body weight of 200 gm). Prolonged administration of cholesterol diet and alcohol will accelerate synthesis of triglycerides, inhibit the metabolism of fatty acids and diminish the secretion of triglycerides from the liver to blood by decreasing the β-oxidation of fatty acids (Luo *et al*., 2009).

5.2 Relative perirenal fat pad mass and kidney function parameter

Faulst *et al*. (1987) stated that perirenal fat pads, which are more responsive to dietary manipulation showed the greatest increase after consuming high-fat diets. Increased perirenal fat in negative control rats fed with high cholesterol diet and alcohol indicating that high cholesterol and alcohol diet will accelerate the accumulation of adipose tissues in the body. These results are in agreement with Abdel-Rahman (2010) who reported that the lipid profile was significantly increased in male rats fed high fat diet supplemented with 1% cholesterol compared with control group. In a study on the interaction of lipid with kidney function by (Trevison *et al*., 2006), lipid-lowering agents seem to ameliorate glomerular damage, preventing glomerulosclerosis and interstitial fibrosis. Oxidative stress, with the resultant increased reactive oxygen species generation, contributed significantly to these chronic degenerative processes. However, in this study, the hypercholesterolaemic state does not induce much kidney injury, as shown by the normal range creatinine and BUN results of all groups (Appendix Table 4). This could be due to limitation of a sub-acute experimental study design.

5.2 Arteriosclerotic index

The arteriosclerosis index (AI) was calculated by the equations as following: AI = $\frac{TC-HDL}{HDL}$. Atherosclerotic index is regarded as a marker for various cardiovascular disorders; the higher the value, the higher the risk of developing cardiovascular disease and vice versa (Hou *et al*., 2009). High-cholesterol and alcohol diet exposure in this specific study resulted in the elevated atherosclerotic index. Oxidative stress is one of the causative factors that link hyperlipidaemia with the pathogenesis of atherosclerosis. An imbalance between free radical production and antioxidant level leads to oxidative stress due to the depressed antioxidant defense system in the negative group of our study.

(Yang *et al*., 2006). The leaves rich in natural antioxidant may prevent atherosclerosis by reducing levels of oxidized lipids in the circulation (Hou *et al*., 2009).

5.3 Non dose-dependent effect

Increasing dose of *Moringa oleifera* hydroethanolic extract did not seem to lead to a pronouncing lipid-lowering effect, as seen in the relative liver weight, relative perirenal fat pad mass and LDL serum level, thus denying the dose effect in this specific study. However, in several previous studies done on the *M. oleifera* leaf extract showed dose-dependent effect. Supplementation with *M. oleifera* methanolic extract at a dose of 400 mg/kg shows a better effect in comparison to 200 mg/kg (Bais *et al*., 2014). Similar results were shown by Ghasi *et al*. (2000), where treatment with crude extract of *M. oleifera* led to an increased serum HDL level and decreased levels of total cholesterol, LDL, and triglyceride in a dose-dependent manner. The non-dose dependent effect in this study could be explained by the short duration of this study and a smaller sample size.

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Thus, from the present study it can be concluded that there is significant effect of the hydroethanolic extract of *M. oleifera* leaves on the serum liver and lipid profile in hypercholesterolaemic rats. The extract promotes reduction in the relative liver weight and perirenal fat pad weight, reduces serum levels of lipids and lipoprotein, abd lowers the atherosclerotic index.

6.2 Recommendation

Further studies can be carry out in order to determine the active principle of this plant, followed by the identification of the mechanistic approach of hydroethanolic extract of *M. oleifera* leaves that helps in feline hepatic lipidosis. Also, longer study period is recommended to induce more pronounced effect of the hypercholesterolaemia and thus more significant comparison. Since the extract was given concurrently with the cholesterol and alcohol diet, making this a preventive study. For further investigation, curative study can be carried out. Among several drugs available for liver injury, silymarin is the most clinically popular for patients and is known to have hepatoprotective and anti-fibrotic properties (Schuppan *et al*., 1999). Therefore, silymarin can be used as a positive control in further study to compare the potency of the extractions.

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APPENDICES

Appendix 1: Effect of *Moringa oleifera* on relative liver weight and relative perirenal fat pad mass between groups.

Group	A	B	C	D	E
Relative liver weight	3.32 ± 0.46	4.11 ± 0.35*	3.79 ± 0.40	3.70 ± 0.44	4.02 ± 0.29
Relative Perirenal Fat Pad Mass weight	1.44 ± 0.66	2.45 ± 0.89*	1.74 ± 0.83	1.34 ± 0.85	1.91 ± 0.10

Value were mean ± SEM (n=5).
*P<0.05, compared with the control group (Group A) (Normal commercial Diet)

Appendix 2: Effect of *Moringa oleifera* on serum liver parameters between groups.

Group	A	B	C	D	E
ALT (U/L)	3.32 ± 0.46	4.11 ± 0.35*	3.79 ± 0.40	3.70 ± 0.44	4.02 ± 0.29
AST (U/L)	1.44 ± 0.66	2.45 ± 0.89*	1.74 ± 0.83	1.34 ± 0.85	1.91 ± 0.10
ALP (U/L)	243 ± 24.54	252.33 ± 10.17	441 ± 30.98*	331.33 ± 23.31	298.5 ± 58.06
Total Protein (g/L)	89.63 ± 3.52	85.83 ± 1.27	92.07 ± 3.37	89.7 ± 5.44	95.6 ± 6.58
Albumin (g/L)	39.57 ± 2.44	37.95 ± 1.35	42.65 ± 2.24	39.23 ± 1.73	45.03 ± 1.44
Globulin (g/L)	50.07 ± 2.06	47.35 ± 0.65	53.95 ± 2.91	50.47 ± 3.89	39 ± 13.52

Value were mean ± SEM (n=5).
*P<0.05, compared with the control group (Group A) (Normal commercial Diet)

Appendix 3: Effect of *Moringa oleifera* on serum lipid profile between groups.

Group	A	B	C	D	E
Cholesterol (mmol/L)	1.8 ± 0.17	1.2 ± 0.1	1.75 ± 0.14	1.43 ± 0.07	1.5 ± 0.17
Triglycerides (mmol/L)	0.65 ± 0.12	0.41 ± 0.06	0.73 ± 0.12	0.54 ± 0.10	0.70 ± 0.10
LDL (mmol/L)	0.51 ± 0.33	1.25 ± 0.05*	0.99 ± 0.16**	0.65 ± 0.41**	0.73 ± 0.22**
HDL (mmol/L)	2.42 ± 0.92	2.85 ± 0.13	3.39 ± 0.22	2.02 ± 0.52	2.91 ± 0.36

Value were mean ± SEM (n=5).
*P<0.05, compared with the control group (Group A) (Normal commercial Diet)
**P<0.05, compared with the negative control group (Group B) (High cholesterol Diet and alcohol)

Appendix 4: Effect of *Moringa oleifera* on creatinine kinase, urea and creatinine between groups.

Group	A	B	C	D	E
Creatinine (umol/L)	64 ± 1.53	62 ± 11	66 ± 3.29	64 ± 4.93	72.5 ± 3.84
Urea (mmol/L)	7.4 ± 1.55	5.4 ± 1.5	6.48 ± 0.39	5.83 ± 0.75	8.13 ± 1.44
Creatinine kinase (mmol/L)	379.67 ± 100.38	347.5 ± 118.5	389.75 ± 159.19	524.67 ± 299.42	896 ± 332.54

Value were mean ± SEM (n=5).
*P<0.05, compared with the control group (Group A) (Normal commercial Diet)